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Pharmacologically Relevant Intake During Chronic, Free-Choice Drinking Rhythms in Selectively Bred High Alcohol Preferring Mice

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Abstract

Multiple lines of High Alcohol Preferring (HAP) mice were selectively bred for their intake of 10% ethanol (v/v) during 24-h daily access over a four-week period, with the highest drinking lines exhibiting intakes in excess of 20 g/kg/day. We observed circadian drinking patterns and resulting blood ethanol concentrations in the HAP lines. We also compared the drinking rhythms and corresponding blood ethanol concentrations (BEC) of the highest drinking HAP lines to those of the C57BL/6J (B6) inbred strain. Adult male and female crossed HAP (cHAP), HAP replicate lines 1, 2, 3, and B6 mice had free-choice access to 10% ethanol and water for 3 weeks prior to bi-hourly assessments of intake throughout the dark portion of the light-dark cycle. All HAP lines reached and maintained a rate of alcohol intake above the rate at which HAP1 mice metabolize alcohol, and BECs were consistent with this finding. Further, cHAP and HAP1 mice maintained an excessive level of intake throughout the dark portion of the cycle, accumulating mean BEC levels of 261.5 ± 18.09 and 217.9 ± 25.02 mg/dl, respectively. B6 mice drank comparatively modestly, and did not accumulate high BEC levels (53.63 ± 8.15 mg/dl). Free-choice drinking demonstrated by the HAP1 and cHAP lines may provide a unique opportunity for modeling the excessive intake that often occurs in alcohol-dependent individuals, and allow for exploration of predisposing factors for excessive consumption, as well as the development of physiological, behavioral, and toxicological outcomes following alcohol exposure.

Keywords

Alcoholism; self-administration; rodent model; ethanol; circadian rhythms; selective breeding

Introduction

Alcohol researchers have striven to model the human condition of alcoholism through developing animal models that model one or more aspects of the disorder. According to

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Author Contributions

LMM and NJG were responsible for the study concept and design, as well as data analysis and interpretation of the findings. LMM and NJG contributed to the acquisition of animal data and drafting of the manuscript. Both authors contributed to the critical review and approved the final version for publication. Neither author has a potential conflict of interest.

Cicero (1980), an animal model of alcoholism should meet five criteria, one of which includes oral administration of alcohol to pharmacologically relevant blood ethanol concentrations. These criteria are related to the symptoms used to define alcohol abuse and dependence (APA, 1994). Researchers have met several of the criteria, but it has been difficult to create a model that successfully meets all of them. Therefore, models exemplify facets of the disorder in an attempt to understand the underlying causes of and develop treatment for alcoholism in humans (Crabbe, Phillips, & Belknap, 2010).

A long history of research in rodents has been aimed at modeling human alcohol consumption. A variety of procedures have been developed to study consumption that have been successful in achieving high levels of intake, although they are often stressful to animals and violate the voluntary aspect of human alcohol consumption (McBride & Li, 1998; Willner, 2005). Preference testing has been widely used to assess alcohol intake in both inbred strains of mice and in selectively bred high and low alcohol-preferring lines of mice and rats (Green & Grahame, 2008). It is a simple procedure, and yields high intake levels as well as information regarding the correlated traits, neurobiology, and the genetics of alcohol preference (McBride & Li, 1998). In addition, the procedure extends a high level of face validity with regard to human alcohol consumption, considering it captures naturalistic consummatory behavior. Further, genetic differences in reinforcement measures appear to be largely captured by assessments of home cage, free-choice alcohol consumption (Green & Grahame, 2008).

There are also limitations to free-choice studies. Pharmacologically relevant BECs have been found in selectively bred P rats, AA rats, sP rats, and the B6 inbred strain, though these levels are relatively modest (about 60-80 mg/dl), and are not sustained in a way that models excessive human intake (Aalto, 1986; Agabio et al., 1996; Dole & Gentry, 1984; Murphy et al., 1986). This makes interpreting the reasons rodents drink difficult, as ethanol consumption may not be for its pharmacologic effects (Cunningham, Fidler, & Hill, 2000; Rodgers et al., 1963). There are individual differences in intake throughout the light-dark cycle, making it difficult to predict periods of high intake and to manipulate or measure behaviors related to alcohol consumption (Crabbe, 2010). Therefore, total daily fluid intake values are reported, which does not lend insight into the relationship between consumption and intoxication, as could be indicated by BECs. Information about intake patterns could potentially aid in acquiring knowledge about the etiology of heavy alcohol intake, as well as allow researchers to better schedule interventions that might reduce drinking.

Recently, scheduled access procedures have been developed that leverage both a genetic predisposition to drink alcohol and the ingestive patterns of rodents to permit high blood ethanol concentrations (BEC) in populations of mice and rats without food or water deprivation (Bell et al., 2006; Rhodes et al., 2005). This is accomplished by providing 2-4 hours of ethanol access when mice or rats are expected to have a high level of intake. These paradigms were developed to control for the variation present in 24-hour, free-choice models; and obtain high levels of intake in a short period of time, resulting in high BECs, meeting the NIAAA definition of binge intake (2004). Therefore, scheduled access paradigms such as Drinking in the Dark (DID) allow researchers to model binge-like intake and to pharmacologically manipulate drinking (Rhodes et al., 2005).

Scheduled-access paradigms have their limitations. First, alcohol access is externally limited, eluding a more naturalistic situation in which animals have constant access to alcohol and other fluids. In addition, the DID procedure takes advantage of the ingestive rhythm of the C57/B6 (B6) mouse strain. Other lines and strains may vary in their circadian drinking patterns, which may affect the degree to which the procedure can be precisely and uniformly applied to any other population of mice, particularly within the same experiment (Aalto, 1986; Jelic, Shih, & Taberner, 1998). Lastly, these methods do not result in the same degree of intake seen in human alcoholics with respect to both timing and amount of alcohol consumption. Although these models are advantageous for modeling binge intake, they are not as desirable for modeling chronic intake.

Selectively bred high alcohol preferring (HAP) mice were selected from the HS/Ibg line (Institute of Behavior Genetics, Boulder, CO) for their intake of 10% ethanol during 24-h daily access over a four-week period (Grahame, Li, & Lumeng, 1999; Oberlin et al., 2010). Recently, we demonstrated that repeated selection of the HS/Ibg progenitor line results in lines that are consistent with respect to the selection phenotype as well as the correlated responses of impulsivity and saccharin consumption (Oberlin et al., 2010; Oberlin & Grahame, 2009). While all of the HAP lines drink considerable quantities of alcohol, the highest intakes are seen in the crossed HAP (cHAP) line, generated by a cross and subsequent selection from HAP replicate 1 (HAP1) \times HAP replicate 2 (HAP2). The other lines drink less, with HAP1 drinking the most followed by the HAP2 and HAP3 lines, respectively.

In the following experiments, the drinking behavior of all of the selected high-drinking lines is characterized by assessing bi-hourly intakes throughout the dark portion of the light-dark cycle. This allows us to observe the ethanol drinking rhythms in all of the HAP lines, and assess when alcohol intakes and corresponding BECs are highest. We hypothesize that the cHAP and HAP lines farther along in the selection process will maintain a higher rate or level of intake throughout the dark portion of cycle. In addition, if these mice maintain a level of intake above the metabolic rate of ethanol, accumulation of blood ethanol should occur throughout the active period.

We were also interested in comparing the drinking rhythms and corresponding BECs of the highest drinking HAP lines to those of the B6 inbred strain, an inbred strain commonly used for modeling high alcohol intake (Belknap, Crabbe, & Young, 1993; Rodgers & Ge, 1962). B6 mice have been observed to consume in excess of about 10 g/kg/day, and it is among the highest drinking inbred strains (Yoneyama et al., 2008). Although B6 mice will consume ethanol to pharmacologic levels, intake occurs in bouts across the dark cycle (McClearn & Rodgers, 1959). Therefore, although we expect the HAP1 and cHAP lines to accumulate high levels of blood ethanol, we expect the B6 mice to maintain a level of intake that will not surpass metabolic capacity, resulting in relatively low BECs.

Materials and Methods

Subjects

Mice were male and female C57BL6/J (B6) and HAP lines of mice born in the IUPUI Animal Care Facilities; parents for the B6 mice were obtained directly from Jackson Laboratories. Two weeks before testing, mice were single-housed in a 12:12 reverse light-dark cycle. Water and food were available ad libitum, and ambient temperature was maintained at $21 \pm 1^\circ \text{C}$. All experiments were performed in drug-naïve mice, and were conducted in the same colony room.

Experiment 1 consisted of 25 male and female HAP3 mice, generation 12, aged 102-115 days upon drinking rhythms analysis. Experiment 2 consisted of 23 male and female HAP2 mice from generations 35 and 36, aged 113-126 days. Experiment 3 consisted of 20 B6 mice, 24 cHAP mice from generation 17, and 23 HAP1 mice from generation 43, with equal numbers of males and females from each mouse population, and aged 69-89 days at analysis of drinking rhythms.

General Procedure

Acquisition

Following their 2-week adaptation period, mice were given 24-hour free-choice access to distilled water and a 10% ethanol (v/v) solution for 3 weeks, and total fluid intake was assessed. We do not report total ethanol intakes during acquisition in HAP2 and HAP3 mice because these have been previously reported (Oberlin et al., 2010). 10% ethanol was used in the following experiments because it is the concentration used during the selection process for the HAP lines. Adult HAP3 and HAP2 mice increase to a stable level of intake following about 3 weeks of ethanol access, which is why this point was used to assess drinking rhythms (Oberlin et al., 2010). We report baseline water intake measured over 2 days, as well as acquisition during 3 weeks of two-bottle choice alcohol access in HAP1, cHAP, and B6 mice. Intakes were measured using 25- or 50-ml graduated cylinders mounted on the wire cage tops. Intakes were recorded and bottles sides were switched 3 times per week.

Drinking Rhythms Following Chronic Alcohol Access

After three weeks of access to ethanol, we continued 24-hour free-choice access to 10 % ethanol (v/v) and water, but used 10 mL stereological pipettes readable to $\pm 0.1 \text{ mL}$. In Experiments 1 and 2, drinking rhythms were assessed for 2 or 3 days from 8 am to 9 pm, with a reading one hour after lights off at 9 am and bi-hourly readings afterwards until 9 pm. In the first two experiments, we observed that HAP mice did not drink to pharmacological levels during the initial hour. Therefore, in experiment 3, we chose to observe mice from 8 am to 8pm for 3 days, with bi-hourly readings beginning at 8 am (lights off). Pipettes were not removed during readings except to refill, and readings were performed as rapidly as possible in order to complete them within 20 minutes.

Blood Ethanol Determination

In Experiments 1 and 2, trunk blood samples were taken at the average highest point of intake during drinking rhythms to assess BECs. In Experiments 1 and 2, an intake reading was taken 1 h prior to sacrifice, and again at the time of sacrifice. In Experiment 1, blood samples were taken 4-5 hours after lights off, corresponding to peak intakes for HAP3 mice. In Experiment 2, there was a broader peak intake period, and we elected to take bloods during the end of that period when BECs would presumably be highest, which also corresponded to the highest mean intake time-point. Therefore, trunk blood samples were taken 7.5-8.5 hours following lights off. During Experiment 3, both lines of HAP mice maintained a stable, high level of intake throughout a majority of the dark period; we chose to sample at the highest average point of intake from the previous 3 days in B6 mice, which also corresponded to the time of day we acquired samples in Experiment 2. Blood ethanol concentrations were assessed using the gas chromatography procedure described by Lumeng and colleagues (1982).

Data Analysis

The alpha level was set at .05. For experiments 1 and 2, a mixed Sex \times Time (8 time points) analysis of variance (ANOVA) assessed whether intake patterns differed between the sexes. Planned comparisons were performed between the highest mean point of intake and all other points of intake, to determine if there was a peak in intake. Data analysis and results from baseline water and ethanol acquisition data are available in the supplementary information. To compare the circadian pattern of ethanol intake between lines during drinking rhythm assessment, we used a mixed Line \times Sex \times Time ANOVA. For experiments 1 and 2, univariate ANOVAs were performed to assess whether there were BEC differences between the sexes; a Line \times Sex ANOVA assessed differences in BECs for experiment 3. For all experiments, because alcohol intakes varied minimally, we collapsed across the multiple days of bi-hourly drinking assessments *a priori* to get the most reliable measure of circadian drinking patterns. In the case of a leak or incorrect reading, the median value for that particular line and sex was used, resulting in less than 1% of the intake scores being imputed for each experiment. In addition, Mauchly's Test of Sphericity was significant for repeated measures ANOVAs ($ps < .05$), therefore we proceeded with the Greenhouse-Geisser test for repeated measures. Spearman's Rho and Pearson correlations were performed to examine the relationship between ethanol intake and BEC. We also report average daily water and ethanol intakes during drinking rhythms assessment, as well as the preference ratios for ethanol in Table 1.

Results

Table 1 indicates overall alcohol and water intakes across all 3 experiments. Alcohol intakes were similar, though slightly lower to values previously reported for the HAP lines. Water intake was generally comparable across the HAP lines, although it was lower in the HAP2 line compared to the other HAP lines. B6 had higher water intake than the HAP lines. Preference ratios for the HAP 2, HAP1, and cHAP lines were extremely high, with ethanol preferences over 90%. HAP 3 mice had slightly lower preference ratios, and B6 mice had the lowest. Total fluid intake was higher in lines that had greater levels of ethanol intake.

Experiment 1: Drinking Rhythms in HAP3 Mice

HAP3 mice demonstrated a peak point of ethanol intake. This was indicated by a Time \times Sex ANOVA performed using the repeated measure Time and Sex, indicating a main effect of Time, $F(4.8, 114.8) = 44.22, p < .001$, and Sex, $F(1, 23) = 5.32, p > .05$. The interaction between these variables was not significant, $F(4.7, 109.1) = 0.92, p < .05$. Post-hoc comparisons were conducted for Time using a Bonferroni correction (.05/8), indicating that intake at the highest point of intake (1-3 pm) was not significantly different from intake between 11-1 pm ($p > .006$), but was significantly higher than all other time-points ($ps < .006$) (Figure 1a). We report all data from drinking rhythms assessment in g/kg/h for clarity in assessing rate of intake across the cycle. A one-way ANOVA indicated there was no difference in mean BEC between the sexes, $F(1, 23) = .833, p > .05$. The correlation between intake and BEC indicated a strong, positive relationship between intake and BEC ($r = .62, p < .001$; $\rho = .70, p < .001$) (Figure 2a).

Experiment 2: Drinking Rhythms in HAP2 Mice

A repeated measures Time \times Sex ANOVA was performed, and indicated there was a main effect of Time, $F(4.6, 96.9) = 35.37, p < .001$, and Sex, $F(1, 21) = 7.57, p < .05$, with mean intakes of 1.04 and 0.88 g/kg/h for females and males, respectively. The interaction between these two variables was not significant, $F(4.6, 96.93) = 1.05, p > .05$. Post-hoc comparisons were conducted for Time using a Bonferroni correction (.05/8 = .006), indicating that intake at the highest point of intake (3-5 pm) was not significantly different from intake between 9-7 pm ($p > .006$), but was different from all the other time-points ($p < .006$) (Figure 1b). A univariate ANOVA indicated there was no sex difference in mean BECs, $F(1, 21) = .521, p > .05$. The correlation between intake and BEC indicated there was a positive relationship between ethanol intake and BEC ($r = .42, p < .05$; $\rho = .50, p < .01$) (Figure 3a).

Experiment 3: Drinking Rhythms in Alcohol-Preferring Mice

During the active period, B6 mice drank at a relatively consistent level that did not result in high BECs, while HAP1 and cHAP mice drank at an excessive level, resulting in high BECs. A repeated measures Line \times Sex \times Time ANOVA was performed on ethanol intake; there were main effects of Line, $F(2, 61) = 8.00, p < .001$, Time, $F(4.8, 293.7) = 103.91, p < .001$, and Sex, $F(1, 61) = 48.69, p < .001$. There were interactions of Line and Sex, $F(2, 61) = 8.00, p < .005$, and Time and Line, $F(9.6, 293.7) = 5.66, p < .001$, but there were no interactions between Sex and Time, $F(4.8, 293.7) = 1.98, p > .05$ or between Line, Sex, and Time, $F(9.6, 293.7) = 0.98, p > .05$. Post-hoc comparisons were conducted by Line for each timepoint using a Bonferroni correction (.05/21 = .002). Ethanol intake in B6 mice at the highest point of intake (2-4 pm) was not significantly different from all points of intake ($p > .002$), except during overnight ($p < .002$). The HAP1 highest point of intake (8-10 am) was only significantly different from intake at 6-8 pm and overnight ($p < .002$), but was not significantly different from all other points ($p > .002$). In cHAP mice, the highest point of intake (8-10 am) was not significantly different from intake during 10-12 and 12-2 pm ($p > .002$), but was different from all other points ($p < .002$) (Figure 1c). A Sex \times Line ANOVA indicated there were significant differences in BEC between the lines and sexes, $F(2,66) = 31.26, p < .00$ and $F(1, 61) = 4.37, p < .05$, respectively, although there was no interaction

between Sex and Line, $F(2, 61) = .340, p > .05$. Females had higher mean BECs than males, reaching 200.23 ± 15.29 and 154.69 ± 15.51 mg/dl, respectively. Post-hoc comparisons were conducted using a Bonferroni correction ($.05/3 = .02$), and indicated that B6 BEC was lower than cHAP and HAP1 ($ps < .001$), but HAP1 and cHAP BECs were not significantly different ($p > .05$) (Figure 4b). The correlation between intake and BEC specified a positive relationship between intake and BEC ($r = .48, p < .001$; $\rho = .59, p < .001$) (Figure 4a).

Discussion

These studies document excessive levels of alcohol drinking within two lines of selectively bred HAP mice. Together, these experiments demonstrate that each HAP line exhibits a predictable and stable pattern of ethanol intake, and importantly, that all HAP lines achieve pharmacologically relevant levels during the active portion of the light-dark cycle. Further, the HAP1 and cHAP lines drank considerably more than the widely used B6 strain, in the absence of water intake differences at baseline, resulting in accumulation of high BECs. Conversely, the BECs we observed in B6 mice are similar to previously published data in a 24-hour free-choice paradigm (Dole & Gentry, 1984). These data suggest that HAP mice, particularly the HAP1 and cHAP lines, may provide an avenue for modeling alcoholism in a highly translational manner. Further, they provide an opportunity to study the “too much, too often” aspect of excessive alcohol consumption in mice that has previously been unattainable (Leeman et al., 2010).

Crabbe (2010) notes that a major problem of two-bottle choice preference testing is that rodents will rarely self-administer ethanol to pharmacological levels. Previous studies in mice have not been able to demonstrate high intake for extended periods of time. Rodents tend to drink in bouts, rarely maintaining levels of alcohol intake above the rate of metabolism (Murphy et al., 1986). This type of drinking is not necessarily translational in nature, in that chronic alcoholics report being unable to control drinking (McKinley & Browne-Mayers, 1968), with higher levels of intake often observed in at-risk and dependent individuals (Dick et al., 2011; Harford et al., 2005). The present studies challenge the notion that with free-choice access, rodents will not self-administer past their capacity to metabolize consumed ethanol. Further, BECs observed in cHAP and HAP1 mice are reminiscent of the excessive levels of intake previously observed in dependent humans using an almost unlimited procedure (Mello & Mendelson, 1970). In this respect, the cHAP and HAP1 lines may provide an opportunity for studying causes, consequences of, and treatments for volitional alcohol consumption.

Patterns of intake are understudied in at-risk and heavy drinking populations (Leeman et al., 2010). These experiments begin to address this issue by shedding light on circadian control of drinking in these lines during a 12:12 light/dark cycle. As might be expected, HAP mice drink at the highest rate during the active portion of the cycle. However, none of the HAP lines drink much more than 1.5 g/kg/hr at any time, but the lines that are further along in the selection process seem to drink at this level during a greater number of hours of the day. This is evidenced by the clear peak time of ethanol intake in HAP3 mice that is close to 1.5 g/kg/hour. This peak broadens incrementally with continued selection in the other lines, such that HAP1 and cHAP lines initiate drinking each day at this high rate, and drinking

remains stable for almost the remainder of the dark cycle. Therefore, greater daily intake is not achieved by increasing hourly intake over this apparent maximum, but by increasing the number of hours per day that a high consumption rate occurs. Further, this expansion may be relatively unidirectional, as cHAP and HAP1 lines seem to “front load” their ethanol, drinking large amounts at lights off. This is similar to chronic alcoholics, who often report drinking upon waking, possibly to alleviate symptoms of withdrawal. Further, alcohol dependent individuals often spend a majority of their waking hours intoxicated (Edwards & Gross, 1976; Stockwell, 1994). We do acknowledge, that the obtained drinking rhythms obtained were under conditions of repeated human intervention, and might not closely resemble the endogenous circadian pattern under undisturbed conditions. Indeed, intakes were somewhat lower than intakes we found when mice were left undisturbed in the home cage. More sophisticated sampling methods, such as those achieved by lick-o-meters may provide additional insight into the natural patterning of ethanol drinking in the HAP lines. Another potential shortcoming for these cross- line comparisons is that we could not run all animals at the same time, and animals from the HAP2 and HAP3 lines were somewhat older. However, intakes we observed here are very similar to those observed during selection of the same lines, in which phenotyping always starts between 45 and 50 days of age (Oberlin et al., 2011).

Further work needs to be done to address the “area under the curve” with regard blood ethanol levels in the HAP lines. We chose to take blood samples at the highest point of intake for B6 mice, despite the fact that cHAP and HAP1 mice continue to drink above the rate of ethanol metabolism for additional hours, suggesting that accumulation of blood ethanol may continue in these lines. Notable is the low variability among cHAP mice in ethanol intake compared to the other HAP lines, and lower variability in BECs than the HAP1 line as is indicated by the SEMs, suggesting that successful prediction of intake and BEC ranges across the day in this line of mice is possible. Quantification of the daily pattern of alcohol exposure in these mice may allow for behavioral, neurobiological, or genetic changes to be examined in a dose- and time-dependent manner.

Consistent daily intake patterns are also useful for investigation of pharmacotherapies for alcoholism. A principle advantage of the DID model is that the timing of intake is predictable because alcohol is presented for 2-4 hours, making it clear when to administer putative medications and allowing for pharmacological modulation of binge drinking (Moore & Boehm, 2009). The predictable and pharmacologically relevant intake patterns during 24-h access in HAP mice suggest that putative medication testing can be timed. Further, observing the time-course of intake following administration of a compound may provide an indication of relative efficacy and duration of action of a pharmacological agent.

A novel aspect of the present work is the ability to explore a new terrain of genetic differences in high-drinking behavior. Figure 5 indicates a steep increase in BEC as daily alcohol intake levels increase over those observed in B6 mice, perhaps because it takes daily alcohol intakes greater than 10-15 g/kg/day to overcome the metabolism of alcohol by the murine liver. Unlike most studies of genetic differences in free-choice alcohol drinking, we demonstrated that the observed populations encountered the pharmacological effects of ethanol, albeit to a varying degree. This increases the probability that intake differences

among these populations are driven by genetic differences related to alcohol's post-ingestive effects.

Investigation of Figure 5 may shed light on an additional issue pertinent to alcohol research. As reiterated by Leeman et al. (2010), diagnosis of alcoholism is categorical in nature. In addition, human studies may examine the number of heavy drinking days, but often do not acquire accurate measurements of the amount consumed. Evident in Figure 5 is that quantitative increases in alcohol consumption across the lines lead eventually to BECs similar to those in alcoholics. This change is presumably caused by gradual accumulation of trait-relevant alleles. Moreover, the daily pattern of alcohol intake, with high rates of drinking beginning at lights out in HAP1 and cHAP lines, seems similar to human abusive drinking patterns. These studies lend support to the idea that quantitative variation in alcohol drinking leads to discontinuity in expression of the disease, the severity of which is presumably associated with the daily alcohol exposure pattern.

In conclusion, HAP lines demonstrate stable, excessive patterns of ethanol intake across the dark portion of the cycle, during which the HAP1 and cHAP mice exhibited intakes considerably higher than B6 mice. Achieving stable, high intake in the HAP mice to the level of intoxication observed provides support for these lines as a rodent model of alcoholism. Further, the excessive intake exhibited by the HAP1 and cHAP lines may provide a unique opportunity for modeling chronic, excessive human intake. Lastly, the excessive intake seen in these lines may allow for exploration into predisposing factors surrounding excessive consumption, as well as the development of physiological and behavioral adaptations outcomes following voluntary alcohol exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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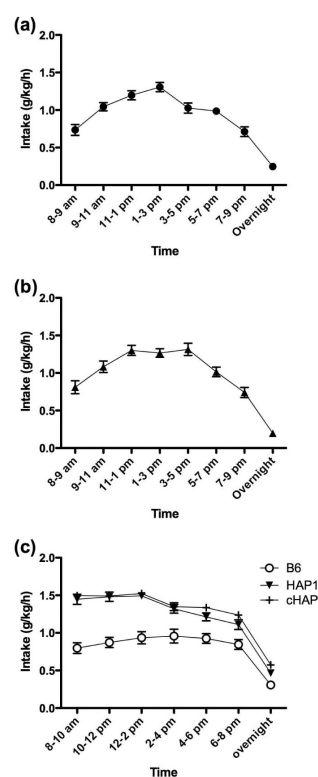


Figure 1.

All intakes are reported in mean g/kg/h. (a) Drinking rhythms in HAP3 mice (b) Drinking rhythms in HAP2 mice (c) Drinking rhythms in B6 (open circles), HAP1 (filled triangles), and cHAP mice (hatches). 95% CIs were constructed for all lines, and indicated that timepoints of intake for each line included the metabolic rate of alcohol, 0.8 g/kg/h, except during overnight. 93×225mm (300 × 300 DPI)

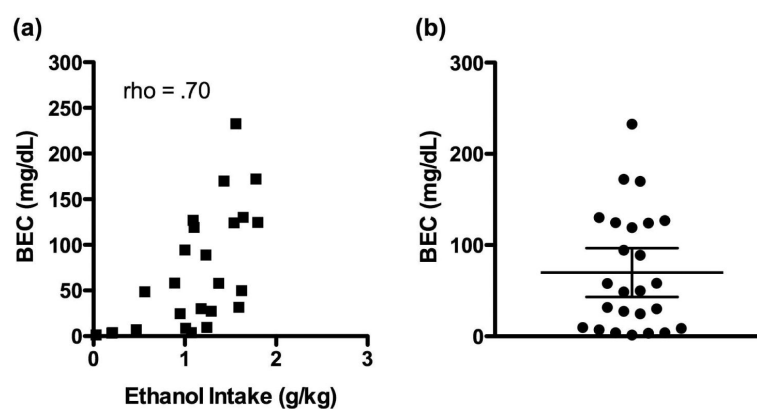


Figure 2.

(a) Spearman's Rho correlation between intake and BEC (b) 95% CI of BEC in HAP3 mice; the mean BEC + SEM was $69.9 + 12.93$ and 40% (10/25) of the mice exhibited BECs above 80 mg/dL. 167×99mm (300 × 300 DPI)

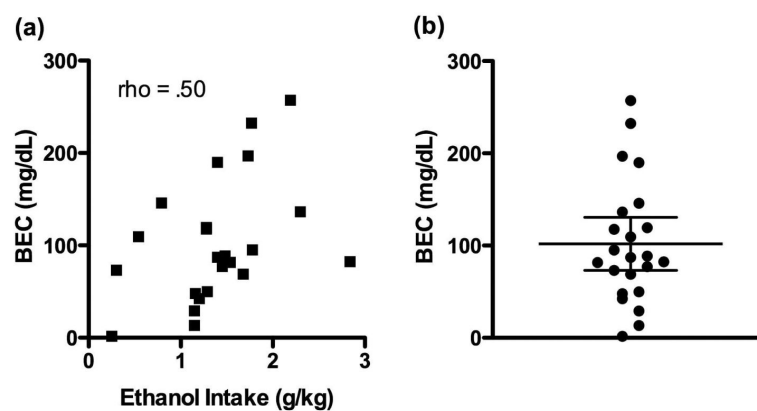


Figure 3.

(a) Spearman's Rho correlation between intake and BEC (b) 95% CI of BEC in HAP2 mice; the mean BEC + SEM was 101.86 ± 13.90 and 68% (14/23) of the mice exhibited BECs above 80 mg/dL. 166×99mm (300 × 300 DPI)

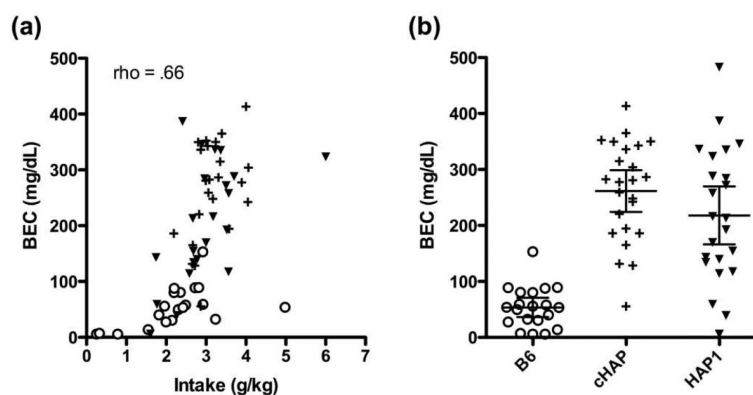


Figure 4.

(a) Correlation between 2-hour intake and BEC in B6, HAP1, and cHAP mice (b) 95% CIs for B6, HAP1, and cHAP mice with mean BECs of 56.63 ± 8.149 , 217.9 ± 25.02 , and 261.5 ± 19.09 , respectively. 30% (6/20) of B6, 87% (20/23) of HAP1, and 96% (23/24) of cHAP mice exhibited BECs above 80 mg/dl. 93×55mm (300 × 300 DPI)

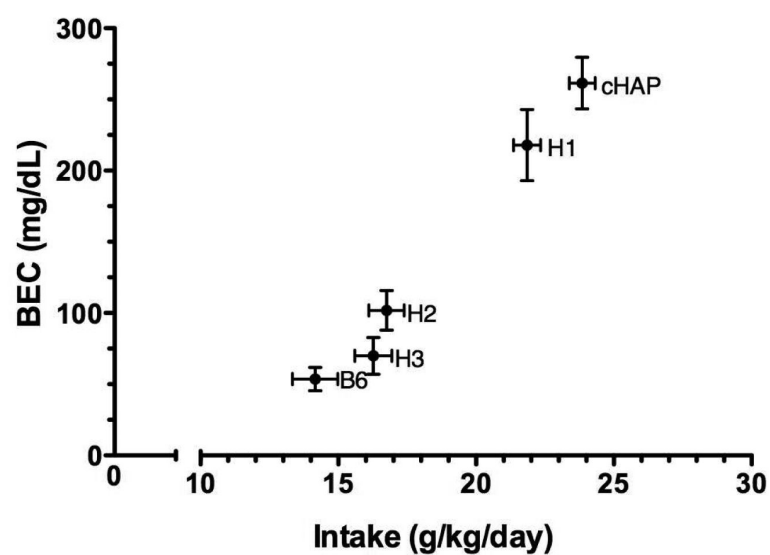


Figure 5. Daily alcohol intakes during the drinking rhythms assessments and corresponding BECs from all 5 populations measured in this series of experiments. 100×80mm (300 × 300 DPI)

Table 1

Average 24-hour intakes of water and ethanol during drinking rhythms across 2-3 days of intake readings, reported in ml/kg/day and g/kg/day, respectively. We also report the mean preference ratios for ethanol during drinking rhythms.

Line	Ethanol Intake	Water Intake	Preference Ratio	Weight	Total Fluid
H3	16.27 ± .67	32.60 ± 4.81	.86 ± .02	27.78 ± .47	6.61 ± .21
Females	17.87 ± .67	33.88 ± 3.60	.86 ± .02	26.65 ± .70	6.97 ± .38
Males	14.77 ± .53	31.43 ± 5.86	.86 ± .03	28.82 ± .51	6.27 ± .17
H2	16.75 ± .67	12.22 ± 2.00	.95 ± .01	28.27 ± .33	6.21 ± .24
Females	17.17 ± .79	13.34 ± 2.51	.94 ± .01	27.22 ± .34	6.57 ± .42
Males	15.56 ± .29	10.99 ± 1.31	.95 ± .01	29.41 ± .34	5.80 ± .14
H1	21.85 ± .49	25.14 ± 2.81	.92 ± .01	23.94 ± .36	7.11 ± .15
Females	23.07 ± .59	23.77 ± 2.50	.93 ± .01	22.75 ± .33	7.17 ± .22
Males	20.51 ± .60	26.62 ± 5.33	.91 ± .02	25.25 ± .39	7.05 ± .21
cHAP	23.85 ± .47	29.44 ± 4.51	.92 ± .01	24.15 ± .43	7.96 ± .16
Females	25.22 ± .58	35.32 ± 6.67	.90 ± .02	22.53 ± .34	8.02 ± .27
Males	22.47 ± .50	23.56 ± 5.86	.93 ± .02	25.78 ± .44	7.90 ± .20
B6	14.16 ± .82	62.52 ± 6.79	.74 ± .03	24.03 ± .47	5.73 ± .18
Females	17.13 ± .69	65.16 ± 10.18	.77 ± .03	22.37 ± .42	6.30 ± .20
Males	11.18 ± .64	59.88 ± 9.45	.71 ± .04	25.69 ± .38	5.16 ± .15